

Co-production of *bla*NDM-1 and *bla*OXA-23 in multiresistant clinical isolates of *Acinetobacter baumannii* from Brazil

Coprodução dos genes *bla*NDM-1 e *bla*OXA-23 em isolados clínicos multirresistentes de *Acinetobacter baumannii* do Brasil

Coproducción de *bla*NDM-1 y *bla*OXA-23 en muestras clínicas multirresistentes de *Acinetobacter baumannii* de Brasil

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Abstract

Acinetobacter baumannii is a common and dangerous non-fermenting gram-negative bacillus due to its various and increasing resistance mechanisms. Therefore, the objective of the work was to describe four NDM-1 -producing *Acinetobacter* isolates from Natal, northeastern Brazil. These four isolates were identified as *Acinetobacter baumannii* by MALDI-TOF. All isolates were characterized by antimicrobial susceptibility testing, with resistance to all β -lactams including carbapenems and positive for the synergy test with Ethylenediaminetetraacetic acid. PCR analysis was also performed for *bla*NDM-1, *bla*VIM-1, *bla*IMP-1, *bla*OXA-23, *bla*OXA-24/40, *bla*OXA-51, *bla*OXA-58, and *bla*OXA-143 genes and showed positive for the *bla*NDM-1 and *bla*OXA-23 genes in all isolates and this result was confirmed by sequencing. This is the first case of *Acinetobacter baumannii* strains harboring *bla*NDM-1 gene isolated from northeastern Brazil. This description emphasizes the need for new prevention and control strategies of the dissemination of NDM-1-producing *Acinetobacter*, which are associated with a high mortality rate.

Keywords: *Acinetobacter baumannii*; *bla*NDM-1; *bla*OXA-23.

Resumo

Acinetobacter baumannii é um bacilo gram-negativo não fermentador comum e perigoso devido aos seus diversos e emergentes mecanismos de resistência aos antimicrobianos. Assim, o objetivo do trabalho foi descrever quatro isolados desta bactérias produtoras do gene NDM-1 em Natal, nordeste do Brasil. Esses isolados foram identificados como *Acinetobacter baumannii* por MALDI-TOF. Pelos testes de susceptibilidade aos antimicrobianos, todas as amostras foram diagnosticadas como resistentes a todos os β -lactâmicos incluindo os carbapenêmicos; também foram positivas para o teste de sinergia com ácido etilenodiaminotetracético. A análise de PCR também foi realizada para os genes *bla*NDM-1, *bla*VIM-1, *bla*IMP-1, *bla*OXA-23, *bla*OXA-24/40, *bla*OXA-51, *bla*OXA-58 e *bla*OXA-143 e mostrou-se positiva para o *bla*NDM-1 e *bla*OXA-23 em todos os isolados, resultado este confirmado por sequenciamento. Destacamos que este é o primeiro caso de cepas de *Acinetobacter baumannii* contendo o gene *bla*NDM-1 isolado do Nordeste do Brasil. Essa descrição enfatiza a necessidade de novas estratégias de prevenção e controle da disseminação da *Acinetobacter* produtora de NDM-1, a qual está associada a uma alta taxa de mortalidade.

Palavras-chave: *Acinetobacter baumannii*; gene *bla*NDM-1; gene *bla*OXA-23.

Resumen

Acinetobacter baumannii es un bacilo gramnegativo no fermentador común y peligroso debido a sus diversos y emergentes mecanismos de resistencia a los antimicrobianos. Así, el objetivo de este trabajo fue describir cuatro aislamientos de esta bacteria productora del gen NDM-1 en Natal, noreste de Brasil. Estos aislados fueron identificados como *Acinetobacter baumannii* por MALDI-TOF. Mediante pruebas de susceptibilidad antimicrobiana, todas las muestras fueron diagnosticadas como resistentes a todos los betalactámicos, incluidos los carbapenémicos; también dieron positivo en la prueba de sinergia con ácido etilendiaminotetraacético. También se realizó análisis PCR para los genes *bla*NDM-1, *bla*VIM-1, *bla*IMP-1, *bla*OXA-23, *bla*OXA-24/40, *bla*OXA-51, *bla*OXA-58 y *bla*OXA-143 y resultó positivo para *bla*NDM-1 y *bla*OXA-23 en todos los aislamientos, resultado confirmado por secuenciación. Destacamos que este es el primer caso de cepas de *Acinetobacter baumannii* que contienen el gen *bla*NDM-1 aislado del noreste de Brasil. Esta descripción enfatiza la necesidad de nuevas estrategias para prevenir y controlar la propagación de *Acinetobacter* productor de NDM-1, que se asocia con una alta tasa de mortalidad.

Palabras clave: *Acinetobacter baumannii*; *bla*NDM-1; *bla*OXA-23.

1. Introduction

The New Delhi metallo- β -lactamase (NDM) enzyme encoded by the *bla*_{NDM-1} gene confers resistance to β -lactams, mainly to carbapenems, except monobactams (Dortet, et al., 2012). The *bla*_{NDM-1} gene was initially identified in *Klebsiella pneumoniae* in New Delhi, India (Yong, et al., 2009). The spread of the NDM-1 variant is described mainly in Asian countries, such as India and China.

In South America, Brazil is the largest reservoir of this variant (Khan, et al., 2017). The highest distribution of this variant is detected in members of *Enterobacteraceae*, but *Acinetobacter* spp. *bla*_{NDM-1} positive reports are increasingly common (Chatterjee, et al., 2016, Joshi, et al., 2017, Pagano, et al., 2015, Pillonetto, et al., 2014, Rozales, et al., 2014, Tran, et al., 2017, Zenati, et al., 2016, Zhang, et al., 2013, Zhang, et al., 2014). In Brazil, there are only few descriptions of *Acinetobacter* spp. NDM-1 producers from the south of the country (Pagano, et al., 2015, Pillonetto, et al., 2014).

The aim of this article is to describe NDM-1-producing *Acinetobacter baumannii* in clinical samples in northeastern Brazil for the first time. Due to the unprecedented nature of the research, we also intend to propose this new possibility to clinicians in the region, reinforcing the problem of bacterial resistance and, thus, generating epidemiological knowledge, essential for better patient care.

2. Methodology

Four carbapenem-resistant *Acinetobacter* spp. were collected between March 2013 and March 2014 from patients admitted to private hospitals in Natal, Rio Grande do Norte. This study was registered at the ethical committee of the Universidade Federal do Rio Grande do Norte at protocol number: 017/2013.

The isolates were identified through conventional biochemical tests, according to technical standard 01/2013 of ANVISA (National Agency for Sanitary Surveillance, 2013) and validated by the MALDI-TOF system (Matrix Assisted Laser Desorption Ionization Time of Flight – BD, Bruker Biotyper, Germany). The strains were tested for susceptibility by the disc-diffusion method (Kirby-Bauer) to gentamicin (GEN), amikacin (AMK), ceftazidime (CAZ), ciprofloxacin (CIP), cefotaxime (CTX), cefepime (CPM), ceftriaxone (CRO), piperacillin + tazobactam (PTZ), sulphamethoxazole + trimethoprim (SUT), ampicillin + sulbactam (APS), tetracycline (TET); imipenem (IMP); meropenem (MER), according to the Clinical and Laboratory Standards Institute (CLSI 2015). The minimal inhibitory concentration (MIC) for polymyxin B (Pol-B) was determined by microdilution broth, according to CLSI (2015) and for tigecycline (TGC) was determined by E-test method (Oxoid, London) according to *Food and Drug Administration* (FDA 2017). The EDTA- modified carbapenem inactivation method (eCIM) was performed for investigation of phenotypic production of metallo- β -lactamases, according to CLSI (2015).

The genes for beta-lactamases *bla*_{NDM-1}, *bla*_{VIM-1}, *bla*_{IMP-1}, *bla*_{OXA-23}, *bla*_{OXA-24/40-like}, *bla*_{OXA-51}, *bla*_{OXA-58-like}, and *bla*_{OXA-143-like} were screened by the Polymerase Chain Reaction (PCR), as described below (Poirel, et al., 2011, Woodford, et

al., 2006). Purification and sequencing (ABI 3730 DNA Analyzer, Life Technologies - Applied Biosystems, Foster City, CA, USA) of PCR products of the *bla*_{NDM-1} gene were identified by comparison with the sequence in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi_NC_020818).

3. Results

All the isolates were identified as *Acinetobacter baumannii* and showed resistance to almost all these antimicrobial classes. They were all sensitive to tetracycline and aminoglycosides with the exception of one isolated (NT 270). They also were all sensitive to polymyxine B and tigecycline and phenotypically positive for Metallo- β -lactamases. The *bla*_{NDM-1}, *bla*_{OXA-51-like} and *bla*_{OXA-23-like} genes were found in all isolates (Table 1) and *bla*_{NDM-1} gene was confirmed by sequencing. The metallo- β -lactamases VIM-1 and IMP-1, as well as Carbapenem-hydrolyzing class D β -lactamases (CHDLs) OXA24/40-like, OXA58-like and OXA-143-like, were not detected in any of the isolates.

Table 1. Resistance features of NDM-1-producing *Acinetobacter baumannii* from Natal.

Isolates	Hospital	Isolation date	Nonsusceptible results (disc diffusion ¹)	MIC		MBL Test (IMP+ EDTA)	Resistance genes							
				TGC (Mg/l)	Pol-B (μ g/ml)		<i>bla</i> _{IMP}	<i>bla</i> _{VIM}	<i>bla</i> _{NDM}	<i>bla</i> _{OXA}	<i>bla</i> _{OXA-51}	<i>bla</i> _{OXA-23}	<i>bla</i> _{OXA-143}	<i>bla</i> _{OXA-24/40}
NT 107.1	B	July 2013	AMK, CIP, CTX, CRO, CAZ, CPM, PTZ, SUT, APS, IMP, MER	1	2	+	-	-	+	+	+	-	-	-
NT 4842	A	August 2013	AMK, CIP, CTX, CRO, CAZ, CPM, PTZ, SUT, APS, IMP, MER	1	1	+	-	-	+	+	+	-	-	-
NT 4917	A	Sept. 2013	CIP, CTX, CRO, CAZ CPM, PTZ, APS, IMP, MER	0.5	2	+	-	-	+	+	+	-	-	-
NT 270	A	March 2014	GEN, AMK, CIP, CTX, CRO, CAZ, CPM, PTZ, SUT, APS, IMP, MER	0.5	0.5	+	-	-	+	+	+	-	-	-

Legend:¹GEN, gentamicin; AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; CPM, cefepime; CRO, ceftriaxone; PTZ, piperacillin + tazobactam; SUT, sulphamethoxazole + trimethoprim; APS, ampicillin + sulbactam; IMP, imipenem; MER, meropenem; TGC, tigecycline; Pol-B, polymyxin. Source: Own autorship (2022).

4. Discussion

Since the first description of *bla*_{NDM-1} gene in 2008 in *Klebsiella pneumoniae* (Yong, et al., 2009), several studies have reported the spread of this gene, with an increasing emphasis on the genus *Acinetobacter* spp. (Adams, et al., 2020, Chatterjee, et al, 2016, Dortet, et al., 2012, Fernández-Cuenca, et al., 2020, Furlan, et al.,2018, Joshi,et al., 2017, Kumari, et al., 2021, Pagano, et al., 2015, Pasteran, et al., 2014, Pillonetto, et al, 2014, Poirel, et al, 2011, Rozales, et al, 2014, Tran, et al., 2017, Woodford, et al., 2006, Zhang, et al., 2013, Zhang, et al., 2014).

In southeastern Brazil, this gene was described for *A. baumannii* in 2014 (Pillonetto, et al, 2014). The presence of the *bla*_{NDM-1} gene in *Acinetobacter* spp. described here is particularly important since the *A. baumannii* species is described as the main disseminator of this gene to other species (Bonnin, et al., 2013) which may spread clones around the world with the risk of overcrowding in hospitals with bacteria with increasingly restricted treatments.

In addition to the NDM enzyme, carbapenem resistance in *Acinetobacter* spp. is also mediated by other metallo- β -lactamases belonging to class B such as VIM-1 and IMP-1 and by CHDLs (Chatterjee, et al., 2016). Although VIM-1 and IMP-1 enzymes have high hydrolytic efficiency against all beta-lactams, except Aztreonam (Héritier, et al., 2005), their

respective genes have not been identified in the isolates. The CHDLs such as OXA-23, OXA-24/40, OXA-58 and OXA-143 contribute significantly to carbapenems resistance and they are epidemiologically more relevant for carbapenem resistance in *Acinetobacter baumannii* (Poirel, et al., 2010).

In this study the *bla*_{OXA-51} gene was detected in all the isolates since it is an intrinsic gene and genetic marker *A. baumannii* species (Higgins, et al., 2009). The *bla*_{OXA-23} was detected in all isolates which was also harbouring the *bla*_{NDM-1} gene. The co-existence of both resistance determinants in the same isolates in this description has been reported in recent studies and they could be independently spread to other gram-negatives population present in the hospital environment (Joshi, et al., 2017, Zenati, et al., 2016) which makes treatment difficult and increases the risk of death from nosocomial infections.

Thus, studies with epidemiological characteristics are essential for monitoring the evolution of resistance in the most diverse locations, especially considering a country with continental dimensions. Additionally, the resistance presented here should serve as a warning for public health authorities to observe and monitor more rigorously its dissemination in other regions.

5. Conclusion

We concluded that the spread of these genes conferring resistance to carbapenems is a great concern because the emergence of multi-resistant microorganisms is a risk factor for nosocomial outbreaks. This description brings attention to the dissemination of carbapenem-resistant strains around the world, reducing the options for treatment of infections by this microorganism. New strategies and public health policies need to be established for the detection and prevention of dissemination of resistant clones, which may compromise the empirical treatments of infections, leading to an increase in morbidity and mortality. Future work should be carried out in other regions of Brazil, seeking to clarify the dissemination of these resistance genes.

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